Supplemental Data. Genre et al. (2008). Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*.



Supplemental Figure 1. Intercellular colonization of the root epidermis by *G. gigantea*. Orthogonal sections (x-z side view) perpendicular to the root axis showing hyphopodium (hp) development between adjacent epidermal cells (e) and elicitation of a transcellular PPA (arrowheads) within the contacted outer cortical cell (oc) in *M. truncatula* (**A**) and *D. carota* (**B**). Bars correspond to $20 \,\mu$ m.



Supplemental Figure 2. Pre-penetration responses in the inner cortex of *M. truncatula* and *D. carota* during AM colonization (see following page for legend).

Supplemental Figure 2. Pre-penetration responses in the inner cortex of *M. truncatula* and D. carota during AM colonization. All the images are z-axis projections of serial confocal optical sections. The plant ER is fluorescently labeled with GFP-HDEL (green) and the autofluorescence of the fungal partner G. gigantea is visualized in yellow-red depending on the depth of the optical section within the root. . (A, B) Intercellular hyphae (ih) in the inner cortex of *M. truncatula*. The inner cortical cells contacted by the fungus all show nuclear (n) repositioning and localized accumulation of ER (arrowheads). The image in (B) shows the increase in size in these repositioned nuclei (n) compared to non-contacted control cells (n'). (C) Composite image (adjacent images separated by vertical dashed lines) of a colonized root of D. carota. Polarized, aligned PPAs within several inner cortical cell files can be oriented either acropetally or basipetally, according to the direction of fungal growth (arrows). (D) Detail showing three inner cortical cells with polarized PPAs in *D. carota*. In addition to the ER large aggregation (arrow) and narrow bridge (arrowhead) described in the legend of Fig. 3, note the fine threads (t) with ER cisternae which are o ften observed connecting the PPA with the cell periphery, possibly anticipating hyphal branching and subsequent colonization of the adjacent cell file. (E, F) Prebranching cytoplasmic aggregations (arrowheads) preceding hyphal branch development within colonized inner cortical cells of D. carota. In (\mathbf{F}) a dichotomous aggregation has assembled in front of the hyphal tip. In these cells nuclei (n) are always located centrally and surrounded by the

broad coiled trunk hyphae. Bars correspond to 20 μ m in (A-C); 10 μ m in (D-F).



Supplemental Figure 3. Ultrastructural studies of *D. carota* inner cortical PPAs: from confocal microscopy to TEM. (A) Confocal image of a series of aligned PPAs in a cell file ahead of the growing hyphal tip. (B) The identical cell file in a semi-thin section (0.5 μ m) imaged by optical microscopy following 1% toluidine blue staining. (C) Ultra-thin section (70 nm) observed by TEM. f = fungus; n = nucleus; m = mitochondrion; p = plastid; arrowhead = Golgi stack. Bars correspond to 5 μ m in (A, B) and 1 μ m in (C).



Supplemental Figure 4. Ultrastructural organization of a control cortical cell from *D. carota*. The nucleus (n) is located peripherally and surrounded by the cell organelles, among which a single Golgi stack can be detected (arrowhead). nu = nucleolus; v = vacuole. Bar corresponds to 2 μ m.



Supplemental Figure 5. Comparison of nuclear architecture in inner cortical cells of *D*. *carota* during different stages of fungal colonization. Nuclear (n) size, shape, degree of chromatin condensation and nucleolar (nu) morphology were remarkably similar in inner cortical PPA-containing cells (right-hand cell in A), cells which had just been infected by an AM hypha (left-hand cell in A), and arbusculated cells (B). f = fungus. Bars correspond to 2 µm.